

In The Claims



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Please amend the claims as follows:

TECH CENTER: 1600/2000

1. (Twice Amended) A method for discrimination and counting erythroblasts comprising the steps of:

(i) staining leukocytes in a hematologic sample by adding a fluorescent leukocyte binding antibody to the hematologic sample to bind the ~~leukocytes~~ ^{leucocytes};

(ii) raising the permeability only of cell membranes of erythroblasts in the hematologic sample to a nucleotide fluorescent dye which does not permeate a cell membrane when the permeability is not raised, the nucleotide fluorescent dye having a fluorescent spectrum that is distinguishable from that of a fluorescent labeling compound of the fluorescent labeled antibody in step (i);

(iii) staining nuclei of the erythroblasts in the hematologic sample with the nucleotide fluorescent dye;

(iv) analyzing the hematologic sample using flow cytometry to detect the nucleotide fluorescent signal of the stained erythroblasts and the fluorescent signal of the labeled antibody ~~[signal of the leukocytes]~~ bound to the leukocytes; and

(v) plotting the nucleotide fluorescent signal and the fluorescent labeled antibody signal in two coordinate axes to obtain a two-dimensional distribution chart discriminating between erythroblasts and leukocytes in the hematologic sample ~~[and counting the erythroblasts from a difference in nucleotide fluorescent signal of the erythroblast and the fluorescent labeled antibody signal of leukocytes]~~ based on the difference in the two-dimensional distribution chart

and counting the erythroblasts [from a difference in nucleotide fluorescent signal of the erythroblast and the fluorescent labeled antibody signal of the leukocytes].

2. (Amended) The ~~[A]~~ method according to claim 1, wherein the fluorescent labeled leukocyte binding antibody ~~[capable of binding specifically with leukocytes]~~ in the step (i) recognizes an antigen present on the leukocytes surface and binds with the antigen.

3. (Thrice Amended) The method according to claim 1 wherein ~~[labeled leukocyte binding the fluorescent leukocyte of]~~ the fluorescent labeled antibody in the step (i) comprises at least one compound selected from the group consisting of phycoerythrin, fluorescein isothiocyanate, allophycocyanin, Texas Red, ~~[CY5 stands for a]~~ arylsulfonate, cyanine fluorescent dye CY5, a peridinin chlorophyll complex, and a combination thereof.

4. (Twice Amended) The method according to claim 1, wherein the raising of the permeability of the cell membranes of erythroblasts in the hematologic sample to the nucleotide fluorescent dye in step (ii) comprises the steps of:

(i) admixing a first reagent fluid of hypotonic osmolarity containing a buffer for maintaining pH within an acidic range to the hematologic sample after the step (i); and

(ii) admixing thereto a second reagent fluid containing a buffer for ~~neutralizing the first reagent fluid containing the hematologic sample and adjusting a mixture of the hematologic sample and the first reagent fluid to a pH wherein the leukocytes are stained]~~ maintaining a pH from 5.0 to 11.0 and an osmolarity compensating agent for adjusting ~~[the mixture to]~~ an osmolarity ~~[suitable for retaining the shape and integrity of the leukocyte]~~ from 300 to 1000 mOsm/Kg H₂O.

5. (Twice Amended) The ~~[A]~~ method according to claim 1, wherein the staining of the nuclei of the erythroblasts in the step (iii) is carried out by mixing the hematologic sample with the nucleotide fluorescent dye.
6. (Amended) The ~~[A]~~ method of claim 5, wherein the nucleotide fluorescent dye comprises at least one compound selected from the group consisting of propidium iodide, N-methyl-4-(1-pyrene)-vinyl-propidium iodide, ethidium bromide, TOTO-1, TOTO-3, YOYO-1, YOYO-3, BOBO-1, BOBO-3, ethidium homodimer-1, ethidium homodimer-2, POPO-1, POPO-3, BO-PRO-1, YO-PRO-1 and TO-PRO-1.

7. (Amended) The [A] method according to claim 1, wherein the nucleotide fluorescent signal of the erythroblasts and the fluorescent labeled antibody signal of the leukocyte [~~at least two fluorescent signals detected from each cell includes a fluorescent signal based on the fluorescent labeled antibody capable of binding specifically with leukocytes and a fluorescent signal based on the nucleotide fluorescent dye and the two fluorescent signals~~] are plotted in two coordinate axes to obtain a two-dimensional distribution chart.
8. (Twice Amended) The [A] method according to claim 1, wherein an area in which the erythroblasts appear is defined on the two dimensional distribution chart and the number of erythroblast cells in the area is ^{counted} ~~connected~~.
9. (Twice Amended) The [A] method according to claim 1, wherein areas in which the leukocytes and the erythroblasts appear are defined on the two-dimensional distribution chart, the number of cells in each of the areas is ^{counted} ~~counted~~ to obtain a leukocyte count and an erythroblast count, and the erythroblast count is divided by the leukocyte count, whereby the ratio of erythroblasts to leukocytes is obtained.

10. (Twice Amended) The method according to claim 5, wherein the nucleotide fluorescent dye is used at a concentration within the range of 0.003mg/L to 10mg/L to form a mixture to be analyzed using flow cytometry to stain erythroblasts [~~according to degrees of maturity of the erythroblasts, and thereby~~] whereby the erythroblasts are classified into at least two groups according to the degrees of maturity thereof.

11. (Amended) The ~~[A]~~ method according to claim 10, wherein:

(i) [(1)] the nucleotide fluorescent signal of the erythroblasts and the fluorescent labeled antibody signal of the leukocytes ~~[at least two fluorescent signals detected for each cell includes a fluorescent signal based on the fluorescent labeled antibody capable of binding specifically with leukocytes and a fluorescent signal based on the nucleotide fluorescent dye and the two fluorescent signals]~~ are plotted in two coordinate axes to obtain a two-dimensional distribution chart;

(ii) [(2)] areas are set in the two-dimensional distribution chart for classifying erythroblasts into at least two groups from difference in intensity of the fluorescent signals based on the nucleotide fluorescent dye; and

(iii) the number of cells in each of the areas is counted for obtaining counts of erythroblasts at different degrees of maturity.

12. (Amended) The ~~[A]~~ method of according to claim 11, wherein an area of all erythroblasts and areas of at least two groups of erythroblasts at different degrees of maturity are defined in the two-dimensional distribution chart, the number of cells in each of the areas is counted to obtain a ~~[sum]~~ total erythroblast count and counts of erythroblasts at the respective degrees of maturity, and the counts of erythroblast at the respective degrees of maturity are divided by the total erythroblast count, whereby the ratios of the erythroblasts at the respective degrees of maturity to all the erythroblasts are obtained.

13. The method according to claim 4 wherein the osmolarity of the leukocytes is about 400 mOsm/Kg.H₂O to about 600 mOsm/Kg.H₂O.